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## Quantitative on-line monitoring of cellular and cerebral energy metabolism

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# 8

## **EVIDENCE FOR A LACTATE POOL IN THE RAT BRAIN THAT IS NOT USED AS AN ENERGY SUPPLY UNDER NORMOGLYCEMIC CONDITIONS**

### **ABSTRACT**

Lactate derived from glucose can serve as an energy source in the brain. It is however not certain how much lactate, directly taken from the blood circulation, may replace glucose as an energy source. This study aims to estimate the uptake, release and utilization of lactate entering the brain from the blood circulation.

The change in cerebral venous-arterial (VA) glucose and lactate differences following lactate infusions in the anesthetized rat was measured. Ultrafiltration probes were placed in the aorta and in the jugular vein, and connected to a flow injection analysis system with biosensors for glucose and lactate. Measurements were taken every minute. At baseline there is lactate efflux, whereas an influx of lactate is seen during lactate infusion. Immediately after the infusion there is net efflux of lactate from the brain. The results suggest that the majority of lactate moving into the brain is not used as an energy substrate, and that lactate does not replace glucose as an energy source. Instead, we propose the concept of a lactate pool in the brain that can be filled and emptied in accordance with the blood lactate concentration, but which is not used as an energy supply for cerebral metabolism.

## INTRODUCTION

It is generally accepted that blood glucose is the major energy substrate of the brain. Only in extreme circumstances other fuel sources may become alternative metabolic energy substrates, e.g. ketone bodies in fasting (Owen et al., 1967; Hasselbalch et al., 1994; Hasselbalch et al., 1996) or lactate in hypoglycemia and brain injury (Maran et al., 1994; Maran et al., 2000; Chen et al., 2000a). Both substances enter the brain via the monocarboxylate transporter (Halestrap and Price, 1999). Inside the brain, lactate derived from glucose may serve as an additional energy substrate. According to the glucose-lactate shuttle hypothesis (Pellerin et al., 1998), glucose is taken up by the astrocytes, where it is converted to lactate to be used as an energy substrate by neurons.

If, however, lactate directly taken up from the blood, can serve as an alternative energy substrate under normoglycemic conditions is not clear. Although the blood-brain barrier (BBB) is readily permeable to lactate (Oldendorf, 1971; Knudsen et al., 1991) and lactate transfer across the BBB can be significant (Lear and Kasliwal, 1991), there is no (Inao et al., 1988; Ide et al., 1999; Ide et al., 2000) or a small net flux of lactate (Frerichs et al., 1990; Madsen et al., 1998; Cruz et al., 1999; Wahren et al., 1999; Leegsma-Vogt et al., 2001; Cruz et al., 1999) under normal resting circumstances, suggesting little cerebral lactate metabolism. Under conditions with an elevated blood lactate level, caused by lactate infusion (LaManna et al., 1993; Dager et al., 1997) or exercise (Ide et al., 1999; Ide et al., 2000), an influx of lactate has been reported, (LaManna et al., 1993; Madsen et al., 1998; Ide et al., 2000) which may indicate that blood-derived lactate is used as a cerebral energy substrate mainly during conditions of increased blood lactate concentration. Studies using radiolabeled lactate infusions have found lactate metabolism in awake mice (Hassel and Brathe, 2000) and the anesthetized rat (Bouzier et al., 2000). Bouzier et al (2000) and Hassel and Br  the (2000) showed substantial lactate metabolism during high blood lactate levels. Using intravenously injected  $[3-^{13}\text{C}]\text{lactate}$ , the cerebral uptake and metabolism of  $[3-^{13}\text{C}]\text{lactate}$  was 50% that of  $[1-^{13}\text{C}]\text{glucose}$  (Hassel and Brathe, 2000), and can contribute up to 35% to cerebral metabolism (Bouzier et al., 2000). Both studies found lactate is almost exclusively metabolised by neurons and hardly at all by glia. If the lactate metabolised by the brain is indeed used as a substitute for glucose metabolism, or as an extra energy source, is not clear.

In the present study, we investigated the change in brain lactate and glucose flux during and after lactate infusions in the rat by monitoring venous-arterial (VA) differences over the brain. We studied the concentration-dependency of the cerebral lactate uptake mechanism, the possibility of lactate metabolism by the brain, and if so, if lactate is used to substitute glucose consumption. Glucose and lactate were measured using intravenous ultrafiltration probes and flow injection analysis (FIA) with biosensors for glucose and lactate. Our approach allows the measurement of glucose and lactate in the aorta and jugular vein simultaneously and every minute, so any change in substrate utilization can be determined nearly on-line (Leegsma-Vogt et al., 2001). The results of the present study suggest little net lactate consumption by the anaesthetized rat brain.

## ANIMALS, MATERIALS AND METHODS

### Animals and surgery

Seven adult male Wistar rats (Harlan, Zeist, The Netherlands) were separately housed on a 12-12 h light/dark regime. The local animal experiments committee (DEC, Groningen, the Netherlands) approved the experiments. Rats were fasted approximately 20 hours before surgery, which was performed on the day of the experiment. The rat was anaesthetized with 30% O<sub>2</sub>, 70% N<sub>2</sub>O combined with halothane (Fluothane, Zeneca, Ridderkerk, The Netherlands), anesthesia was maintained throughout the experiment. The rat was tracheotomised (Insyte iv catheter, 14 G, 2.1 x 45 mm, Becton Dickinson, Sandy, Utah, USA) for mechanical ventilation (MK2 infant ventilator, Hoek Loos, Loosco, Amsterdam). For heparin administration and lactate-infusion, a needle (0.6x25 mm, 23G x 1", Braun, Melsungen, Germany) with the conus removed was placed in the tailvein and connected via a fine bore polyethylene tubing (0.28 mm ID, 0.61 mm OD) of about 40 cm to a syringe filled with saline.

VA differences were measured by placing one ultrafiltration probe (see below) in the aorta and another in the right jugular vein near the bifurcation with the external jugular vein. The aorta was mainly chosen for its relatively large diameter. We assume that the concentration of glucose and lactate in the aorta is equal to the concentration entering the brain via the carotid artery. The jugular vein is the main effluent vein of the brain, easily accessible and large enough for probe-placement. We are aware that the jugular vein also carries blood from the head and front paw, but in the anesthetized animal, contribution in the blood glucose and lactate levels is expected to be minimal. To minimize blood-flow disturbances, we placed the probes in the direction of the blood flow. In previous experiments, the potential of the two-probe system for the measurement of brain arterio-venous measurements was demonstrated (Leegsma-Vogt et al., 2001). After probe-placement, the blood vessel was sealed with cyano-acrylic glue (Ruplo, Ten Boer, The Netherlands). The left jugular vein was ligated to force a higher blood flow through the probed vein. The rats were heparinised through the tailvein after the insertion of the aorta-probe (50 IU/ml saline, Leo Pharma B.V., Weesp, The Netherlands).

In 4 animals, a cannula (5 mm length of fine bore polyethylene tubing, 0.28 mm ID, 0.61 mm OD, connected to 10 cm length silicon tubing, 0.5 mm ID 1 mm OD) was placed in the femoral artery and connected via a blunted needle (0.6x25 mm, 23G x 1", Braun, Melsungen, Germany) to a syringe for blood withdrawal. Four times during the experiment, approximately 150 µl blood was taken for the measurement of blood pH, pO<sub>2</sub>, pCO<sub>2</sub>, oxygen saturation and base excess, all measured at once with a portable clinical analyzer (I-STAT, cartridge type G3<sup>+</sup>, Abbott, Wiesbaden-Delkenheim, Germany). The blood gas values were monitored during baseline situations, immediately after the lactate infusion, and at the end of the experiment. In three rats two baseline measurements were taken, in one rat there was an extra measurement after the lactate infusion. The average pH and blood gas values were calculated. After surgery, the animal was transferred and connected to the Flow Injection Analysis (FIA) apparatus (description below).

### Ultrafiltration probe, flow injection analysis system and biosensors

The ultrafiltration probe, the FIA system and the biosensors used in these experiments are essentially the same as described earlier (Leegsma-Vogt et al., 2001). In short, the probe consists of a filtration membrane (acrylonitrile-sodium methallyl sulfonate copolymer, Filtral 16; Hospal Ind., Meyzieu, France, outer diameter = 290  $\mu\text{m}$ , inner diameter = 240  $\mu\text{m}$ , molecular weight cut-off 20kD) connected to a 15-20 cm long fused silica tube (inner diameter 50  $\mu\text{m}$ , outer diameter 150  $\mu\text{m}$ , Polymicro Technologies, Phoenix, AZ, USA). After the insertion of the probe in the aorta and the jugular vein, the open side of the fused silica tubes is connected to the FIA system. By applying underpressure, blood ultrafiltrate is pulled through the probe towards the FIA system and the biosensors at a flow of 100 nl/min. The two fused silica tubes (one from the jugular vein, one from the aorta) are each connected to the analytical part of the set-up by an intercalated valve. The valve with a 20 nl internal loop (Vici-Valco Instruments, Houston, USA) injects a 50-s collected ultrafiltrate sample every minute. Glucose and lactate are measured using glucose oxidase and lactate oxidase respectively, placed in four separate enzyme reactors together with horseradish peroxidase (all enzymes from Roche, Mannheim, Germany). The resulting current is measured using wall-jet-type electrochemical flowcells (VT-03, Antec Leyden B.V., Zoetermeer, The Netherlands) and four potentiostates (two Decades (Antec Leyden B.V., Zoetermeer, The Netherlands), an Amor amperometric detector (Spark Holland, Emmen, The Netherlands), and a home-made potentiostate (Central electrical service, Groningen University, Groningen, The Netherlands)). A schematic representation of the experimental set-up is shown in figure 8-1a.

During the surgery, an in vitro calibration curve is run on the FIA (glucose: 0, 2.5, 5, 10, 20 mM; lactate: 0, 1.25, 2.5, 5 and 10 mM). During the experiment, the glucose and lactate concentration is measured every minute and the currents (in nano-amperes) are recorded on two double-pens recorders (BD 112, Kipp en Zonen, Zoetermeer, The Netherlands) and by a data acquisition program (Chromeleon, Dionex Corporation, Sunnyville, CA, USA).

### Experimental procedure

First, a baseline is recorded for at least 30 minutes. Then lactate (sodium-L-lactate, 1 M, pH 7.4, dissolved in potassium-phosphate buffered saline (KPBS)) is infused at a rate of 100  $\mu\text{l}/\text{min}$  for different time-periods (5-33 minutes) to achieve various maximal lactate levels. One experiment was done at an infusion rate of 50  $\mu\text{l}/\text{min}$  for 80 minutes to reach a steady state during lactate infusion. After the infusion the blood lactate level was allowed to return to baseline. The rats were killed at the end of the experiment.

### Calculations

As only about 80% of the blood volume consists of water, glucose molarity of whole blood is different from the ultrafiltrate molarity (Marks, 1996). To make correct comparisons with whole blood glucose determinations, we adjusted the ultrafiltrate concentrations by -15% to unite with clinical practice of glucose molarity measurements in whole blood (Tiessen et al., 2001). As the exchange over the erythrocyte-membrane is much higher than the glucose use by the erythrocytes (Jacquez, 1984) haematocrite values are ignored. Erythrocytes do not have mitochondria to aerobically break down metabolic products; they acquire energy

through anaerobic glycolysis. We assume that the erythrocytes do not actively participate in the lactate pool in contrast to their role in glucose metabolism, as the typical lactate concentration in erythrocytes is higher than the normal lactate concentration in the blood (normal lactate concentration in blood: 1-1.5 mM, erythrocyte lactate concentration: 2.9 mM, (Stryer, 1995)) and the maximal transport-rate of the MCT1 transporter, present on erythrocytes, in adult rats is not very high (0.023  $\mu\text{mol/g/min}$  (Cremer et al., 1979); 0.97  $\mu\text{mol/g/min}$ , (Kuhr et al., 1988)). Therefore, we corrected the lactate values by -50% to correct for haematocrite for comparisons with whole blood lactate determinations.

For both glucose and lactate, averages  $\pm$  SD were calculated during baseline (calculated average over 30 minutes), during lactate infusion (calculated average over infusion time) and after lactate infusion (calculated average over 60 minutes), for the aorta, the jugular vein and the venous-arterial differences (table 8-2). These averages were used for the calculation of correlation between the aorta lactate concentration and the flux of lactate, and for the correlation between the VA glucose with the VA lactate difference.

From the deltas of the venous-arterial difference during infusion and after infusion (thus minus baseline), the area under the curve (AUC) was calculated as the sum of the minute-to-minute delta concentrations. We assumed the curve to be divided into one-minute-wide columns, in which the AUC is the sum of the height of these columns. The AUC during infusion was calculated over the length of the infusion time, the AUC after infusion was taken over a period of 60 minutes.

## Statistical Analysis

Correlations were calculated between the aorta lactate concentration and VA lactate, between the VA glucose concentration and VA lactate concentration, and between the AUC during infusion and the AUC after infusion. Differences between baseline, during infusion and after infusion, for aorta, jugular vein, or VA glucose or lactate levels, and differences in glucose fluxes in separate lactate flux groups (lactate influx, no lactate flux, lactate efflux) were analyzed with ANOVA with a bonferroni post-hoc test. Values are given as mean  $\pm$  SD.

## RESULTS

### Experimental examples

An example of an experimental printout and the graphic representation of an entire experiment are presented in figures 8-1b and 8-1c respectively. In figure 8-1b, two separate calibration curves are shown, as well as a part of the curves with the original lactate (above) and glucose (below) measurements. The minute-to-minute peaks have a recurrent return to baseline. Insets show straight-line calibration-curve plots, current vs. concentration. The equations of the regression lines are: glucose (right plot):  $y=0.21x + 0.13$ ,  $R^2=0.9998$ , lactate (left plot):  $y=0.021x - 0.92$ ,  $R^2=0.9978$ .

After correcting the data (see Methods) and calculating the VA differences, connecting the minute-to minute measurements creates a graph as shown in figure 8-1c. Arrows indicate infusion start and ending. Before lactate infusion, we measured stable baseline levels for at

least 30 minutes. During all baseline measurements, there was glucose influx and lactate efflux.

**Physiologic variables**

Baseline glucose levels are in the normal range. After lactate infusion the glucose concentration is significantly elevated compared to baseline, both in the aorta ( $p=0.012$ ) and jugular vein ( $p=0.003$ ). In the jugular vein, the glucose concentration after infusion is significantly elevated compared to during infusion ( $p=0.046$ ). However, glucose VA differences do not change significantly during the course of the experiment (figure 8-1c, table 8-2). During lactate infusion, lactate influx is seen, which turns into an even higher than baseline efflux immediately after lactate infusion, and then returns to stable VA lactate levels (figure 8-1c, table 8-2). The lactate levels during infusion are significantly different compared to baseline and after infusion, in the aorta (during infusion vs baseline:  $p=0.005$ , during infusion vs after infusion:  $p<0.000$ ), in the jugular vein (during infusion vs baseline:  $p<0.000$ , during infusion vs after infusion:  $p<0.000$ ), and in the VA differences (during infusion vs baseline:  $p<0.000$ , during infusion vs after infusion:  $p=0.021$ ).

Of 4 animals, the pH and blood gas was analyzed (table 8-1). The baseline pH was somewhat above normal range (normal arterial range: 7.35-7.45, all reference ranges provided by I-STAT) and the baseline CO<sub>2</sub> below normal range (normal arterial range: 4.67-6.0). Perhaps the used infant ventilator may have caused these deviations, as these ventilators are not ideally suited for use on small laboratory animals. The baseline pO<sub>2</sub> was somewhat above normal range (normal arterial range: 10.7-14.0), but stabilized during the experiment. The baseline HCO<sub>3</sub> and base excess were within range (normal range HCO<sub>3</sub>: 22-26, normal range base excess: -2 - +3). During infusion, the pH, HCO<sub>3</sub> and base excess rise and remain high during the remainder of the experiment.

Average blood gas and pH values $\pm$ SD, n=4	pH	pO <sub>2</sub> (kPa)	pCO <sub>2</sub> (kPa)	HCO <sub>3</sub> (mM/L)	BE <sub>ecf</sub> (mM/L)
Baseline	7.50 $\pm$ 0.03	14.79 $\pm$ 2.81	3.83 $\pm$ 0.48	22.5 $\pm$ 1.1	-0.8 $\pm$ 1.0
After infusion	7.59 $\pm$ 0.05	12.55 $\pm$ 2.38	3.87 $\pm$ 0.59	27.6 $\pm$ 3.6	6.1 $\pm$ 3.8
End	7.56 $\pm$ 0.05	13.10 $\pm$ 2.45	4.07 $\pm$ 0.56	27.3 $\pm$ 3.6	5.3 $\pm$ 4.0

Table 8-1: Blood gas and pH values ( $\pm$  SD) before, during and after lactate infusion. Both the baseline pH and pO<sub>2</sub> were somewhat above the normal range (normal arterial range: pH: 7.35-7.45, pO<sub>2</sub> 0.7-14).The baseline CO<sub>2</sub> was below normal range (normal arterial range: 4.67-6.0). The pO<sub>2</sub> stabilized during the experiment. The baseline HCO<sub>3</sub> and base excess were within range (normal range HCO<sub>3</sub>: 22-26, normal range base excess: -2 - +3). During infusion, the pH, HCO<sub>3</sub> and base excess (BE<sub>ecf</sub>) rise and remain high during the remainder of the experiment.

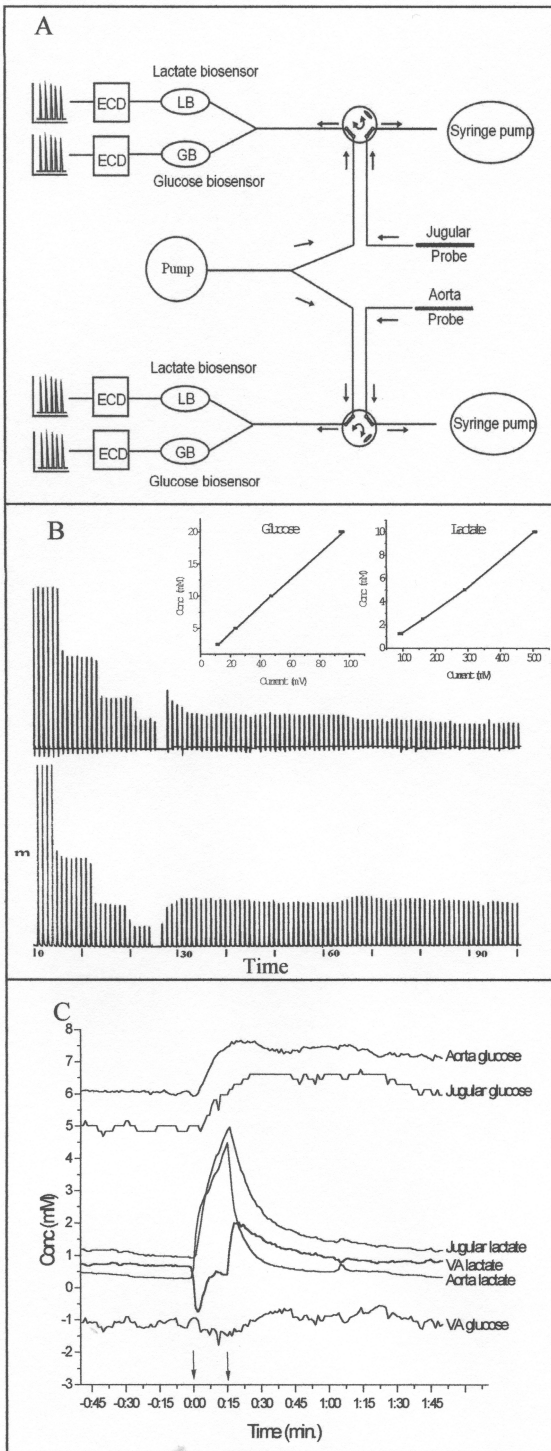


Figure 8-1 A, B, C: Figure 8-1a shows a schematic set-up of the flow injection analysis (FIA) system. Arrows indicate flow direction. The flow in the ultrafiltration probe is 100 nl/min. ECD = electrochemical detection, LB = lactate biosensor, GB = glucose biosensor. An example of an experimental printout is shown in figure 8-1b, with a calibration curve (left) and part of the measured rat ultrafiltrate glucose (below) and lactate (above) concentrations. Insets show straight-line calibration-curve plots, current vs. concentration. The equations of the regression lines are: glucose (right plot):  $y=0.21x+0.13$ ,  $R^2=0.9998$ , lactate (left plot):  $y=0.021x-0.92$ ,  $R^2=0.9978$ . Ultrafiltrate samples are measured every minute, with a recurrent return to FIA-baseline. The time-period in minutes is presented on the x-axis. Connecting all minute-to minute samples creates a graphical representation as shown in figure 8-1c. Arrows indicate infusion start and ending. There is glucose influx throughout the experiment. Before lactate infusion there is lactate efflux, which turns to influx during infusion. Directly after the lactate infusion lactate efflux is higher than before infusion.



Average $\pm$ SD glucose concentration (mM, n=5)	Aorta	Jugular vein	VA difference
Baseline	6.13 $\pm$ 0.52	5.57 $\pm$ 0.50	-0.55 $\pm$ 0.35
During infusion	6.92 $\pm$ 0.73	6.07 $\pm$ 0.55	-0.85 $\pm$ 0.46
After infusion	7.54 $\pm$ 0.61	6.95 $\pm$ 0.45	-0.58 $\pm$ 0.30
Average $\pm$ SD lactate concentration (mM, n=8)	Aorta	Jugular vein	VA difference
Baseline	0.42 $\pm$ 0.06	0.71 $\pm$ 0.07	0.29 $\pm$ 0.07
During infusion	3.47 $\pm$ 0.43	2.85 $\pm$ 0.34	-0.62 $\pm$ 0.19
After infusion	0.97 $\pm$ 0.23	1.64 $\pm$ 0.36	0.67 $\pm$ 0.15

Table 8-2: Average glucose and lactate concentrations at baseline, during lactate infusion and after infusion. Baseline glucose levels are in the normal range. After lactate infusion the glucose concentration is significantly elevated compared to baseline, both in the aorta ( $p=0.012$ ) and jugular vein ( $p=0.003$ ). In the jugular vein, the glucose concentration after infusion is significantly elevated compared to during infusion ( $p=0.046$ ). Glucose VA differences do not change significantly during the course of the experiment. The lactate levels during infusion are in all cases significantly different compared to baseline and after infusion. Values are in mM  $\pm$  SD, n=5.

### Lactate flux and metabolism

From the correlation between the mean aorta lactate concentration during baseline, infusion and after infusion and the accompanying VA lactate (figure 8-2a, n=8,  $r=0.63$ ,  $p=0.001$ ), it can be shown that a lactate efflux turns to an influx at higher aorta lactate concentrations. The zero net flux point is approximately 1.75 mM.

The lactate AUC during infusion and the lactate AUC after infusion are significantly correlated (figure 8-2b, n=8,  $p=0.01$ ). All experiments showed an influx of lactate during the infusion, and an efflux of lactate after the infusion. Thus, the amount of lactate moving into the brain during infusion correlates to the amount of lactate leaving the brain after infusion. The calculated function (see figure 8-2b) reveals an almost 1:1 correlation ( $y = 0.89x$ ). Glucose data from five lactate infusion experiments were used for glucose uptake calculations. Averages were calculated for baseline, during infusion and after infusion. Average glucose use can be calculated, taking a CBF rate from other halothane-anesthesia experiments (150 ml/100g/min, average of two studies: (Verhaegen et al., 1992; Linde et al., 1999)) and a perfused brain-area of 1 gram. The baseline VA glucose differences give a glucose use of 0.83  $\mu\text{mol/g/min}$ . Correcting the cerebral metabolic rate for glucose ( $\text{CMR}_{\text{glc}}$ ) for the lactate efflux during baseline ( $-0.12 \pm 0.16$ , assumed to be derived from glucose) gives a  $\text{CMR}_{\text{glc}}$  of  $0.83 - 0.09 = 0.74 \mu\text{mol/g/min}$ . Assuming a constant brain metabolism rate during the experiment, less glucose will be used when lactate replaces glucose as energy substrate, e.g. during high circulating lactate levels. If lactate were metabolized, a negative correlation would be expected between VA differences of lactate

and glucose. In our experiments however, no correlation is found between glucose and lactate flux in as well an inter-animal comparison (figure 8-2c) as an intra-animal comparison (data not shown). Another way of analyzing lactate metabolism is by comparing the average glucose flux for different lactate fluxes. Therefore, we divided the lactate flux into three groups: lactate influx (average:  $-1.17 \pm 0.39$ ,  $n=5$ ), (almost) no lactate flux ( $0.18 \pm 0.11$ ,  $n=5$ ) and lactate efflux ( $0.96 \pm 0.27$ ,  $n=5$ ) and compared the concomitant glucose flux (glucose at lactate influx:  $-0.68 \pm 0.40$ , glucose at no lactate flux:  $-0.51 \pm 0.49$ , glucose at lactate efflux:  $-0.75 \pm 0.26$ ). When lactate is metabolized, the average glucose influx should be different between the separate groups. But, again, no differences were found between the glucose fluxes in the different groups ( $F= 0.504$ ,  $p=0.617$ ) and thus, no evidence is found that lactate replaces glucose as metabolic substrate under the experimental conditions.

## DISCUSSION

### Some methodological aspects

Lactate infusions have been used in cerebral studies to assess uptake (Dager et al., 1992a; Dager et al., 1992b; Chen et al., 2000a; Chen et al., 2000b) and metabolism (Hassel and Brathe, 2000; Bouzier et al., 2000). Elevated lactate levels as found in our study are within physiological range found during exercise in humans (Ide et al., 2000) and rats (Fregosi and Dempsey, 1984). The present report is based on measurements with a high time resolution of AV differences of lactate and glucose over the anaesthetized rat brain under non-pathological conditions. The current approach allows 4 simultaneous measurements every minute, resulting in almost 1000 values in a 4-hour experiment. Glucose and lactate levels are both measured in the same sample, and the aorta and jugular vein blood levels are monitored at the same time. Central elements in the set-up are ultrafiltration and biosensor technology. Ultrafiltration sampling does not require complicated calculations of the recovery, as the recovery for glucose and lactate is 100% and remains constant during the experiment. The combination of flow-injection analysis and biosensor technology has already been used in several other studies (Kaptein et al., 1997; Rhemrev-Boom, 1999; Tiessen et al., 1999; Tiessen et al., 2001; Leegsma-Vogt et al., 2001; Savenije B et al., 2002). For details concerning specificity, sensitivity and speed of the lactate and glucose assays, we refer to our previous reports.

We considered the (confluence of the) sinus, being the direct afferent vein of the cerebral cortex, as a site for the placement of the ultrafiltration-probe alternative to the jugular vein used in the present study. Unfortunately, the sinus is too short for the ultrafiltration-probes and we considered the risk of bleeding too high. Therefore we preferred the jugular vein for probe implantation. The jugular vein carries blood from extra-cerebral tissue, such as the facial muscles and the front limb, as well. During anesthesia, these contributions are small as compared to those of the brain and are therefore ignored. Evidence that the brain does indeed contribute to jugular venous lactate was reported previously, as jugular lactate was affected by acute brain injury (Leegsma-Vogt et al., 2001).

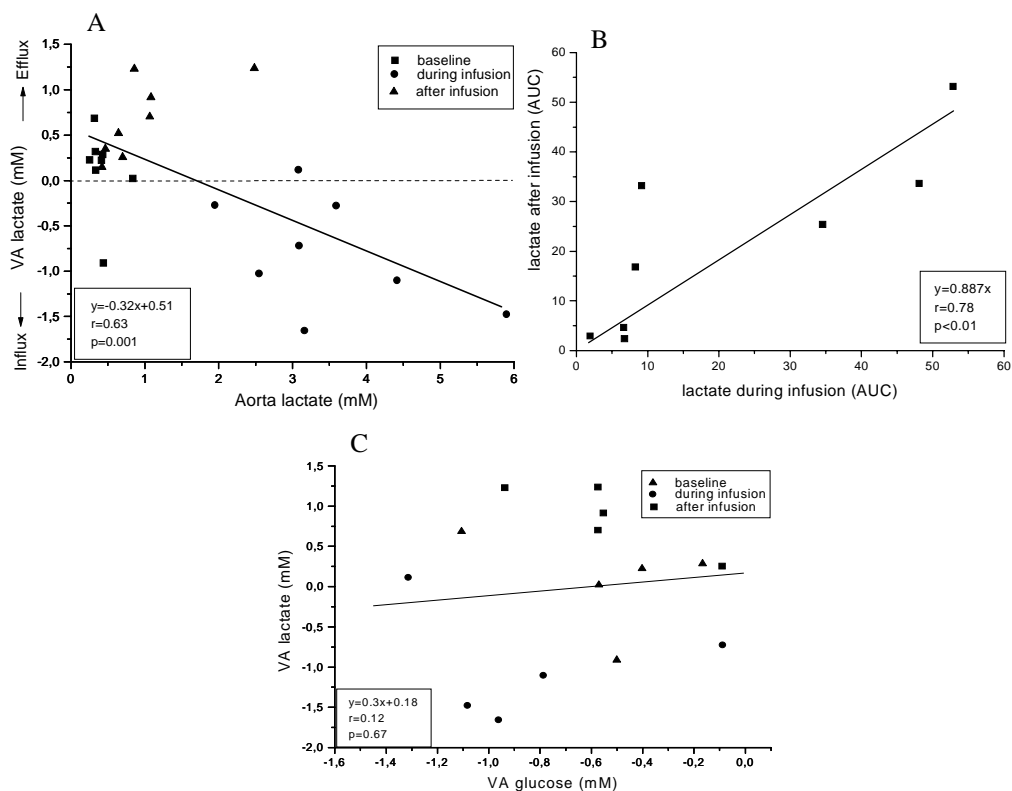


Figure 8-2 A, B, C: Figures a, b, and c are all inter-animal comparisons. Figure 8-2a shows the correlation between the mean blood lactate concentration during baseline, infusion and after infusion, and the accompanying flux of lactate. The lactate flux is dependent on the arterial lactate concentration ( $r=0.63$ ,  $p=0.001$ ). During “normal” blood lactate levels a lactate efflux is seen, at blood lactate levels above approximately 1.75 mM, there is lactate influx. The horizontal dashed line indicates zero net flux. Figure 8-2b: The amount of lactate moving into the brain during infusion is correlated to the amount of lactate coming out of the brain after infusion ( $n=8$ ). The area under the curve (AUC) during infusion correlates with the AUC after infusion with an almost 1:1 relation ( $y=0.89x$ ), suggesting that most of the lactate moving into the brain during infusion is released from the brain after infusion. Figure 8-2c: A comparison between the average VA glucose levels during baseline, during infusion and after infusion and the corresponding VA lactate levels ( $n=5$ ). Glucose- and lactate flux are not correlated, implying that lactate does not replace glucose in brain metabolism.

High lactate disturbs the acid-base balance (Hood and Tannen, 1998). High lactate may initially cause a low blood pH, followed by an alkalization due to aerobic metabolism (Gladden and Yates, 1983). Above an infusate pH of 4.4, alkalization predominates (Gladden and Yates, 1983); optimal respiration may reduce the alkalization. Alkalosis, as seen in our experiments, could decrease lactate production (Buchalter et al., 1989),

clearance (Hetenyi, Jr. et al., 1988) and transport across the BBB (Oldendorf et al., 1979). Decreased lactate production and clearance will be equal in the aorta and jugular vein and thus will not influence our VA conclusions. The effect of lactate on CBF is in our opinion unresolved: one study in lactate induced panic patients showed a 20% increase in CBF in normal control subjects (Stewart et al., 1988). However, another study found no significant effect of lactate on CBF in non-panicking patients or control subjects (Reiman et al., 1989). Any possible increase in CBF will have no effect on the VA glucose and lactate levels, as the increase in CBF will be equal in the aorta and the jugular vein because the brain is unable to expand in volume, and one jugular vein is ligated, forcing all jugular blood via the probed jugular vein. Halothane does probably not influence lactate metabolism directly, as halothane does not affect lactate dehydrogenase activity (Johnstone et al., 1976) and lactate metabolism in hepatocytes (Becker, 1990). However, cerebral metabolism is reduced during halothane anesthesia (Sibson et al., 1998; Alkire et al., 1999; Attwell and Laughlin, 2001), and CBF increased (Verhaegen et al., 1992; Kuroda et al., 1997; Linde et al., 1999; Paut and Bissonnette, 2001).

### **Glucose metabolism**

The average cerebral metabolic rate for glucose (CMR<sub>glc</sub>) in our experiments is 0.74  $\mu\text{mol/g/min}$ . As the CBF is increased during halothane anesthesia (Verhaegen et al., 1992; Kuroda et al., 1997; Linde et al., 1999; Paut and Bissonnette, 2001), we have used an average CBF under halothane anesthesia (averaged from (Verhaegen et al., 1992; Linde et al., 1999)) of 150 ml/100g/min and a perfused brain area of 1 gram. The calculated CMR<sub>glc</sub> is slightly above range of CMR<sub>glc</sub> values under anesthesia reported in literature (40-69  $\mu\text{mol/100g/min}$ ) (Mies et al., 1990; Verhaegen et al., 1992; Ueki et al., 1992), which could be due to altered hemodynamics because of the ligation of one jugular vein (Chai et al., 1995). For the comparison of VA differences, which is the main objective of this article, the exact measurement of CBF values is less important, as the blood flow entering the brain is equal to the blood flow going out of the brain due to the physical expansion barrier caused by the skull, and due to the ligation of one jugular vein. After the lactate infusion both arterial and venous glucose concentrations are significantly elevated, although the VA glucose difference is not significantly changed. This increase in blood glucose may be caused by gluconeogenesis from lactate by the liver (Bouzier et al., 2000; Brooks, 2000).

### **Lactate uptake, release and brain metabolism**

Influx of lactate has been observed in the human and rat during exercise and it was suggested that the uptake of lactate is dependent upon enhanced activity of the brain (Ide et al., 2000). We used anesthetized rats, with a diminished brain activity, so we conclude that lactate uptake does occur irrespective of the state of the brain, which was also shown in our previous article (Leegsma-Vogt et al., 2001).

The lactate flux is concentration dependent; at normal levels of lactate there is lactate efflux, at higher blood lactate levels there is lactate influx. In this study the equilibrium-point is approximately 1.75 mM, at which the flux of lactate into the brain is equal to the amount of lactate leaving the brain. This equilibrium-concentration is somewhat higher than in our previous experiments (Leegsma-Vogt et al., 2001), but still in between the  $K_m$  values of the monocarboxylate transporters (MCT1: 3.5 mM, MCT2: 0.5 mM, (Broer et al., 1998; Broer et al., 1999)).

There is a significant correlation between the amounts of lactate taken up by the brain during infusion and released from the brain after infusion: almost all of the lactate taken up during infusion is released afterwards (89%). This indicates that 11% of the lactate taken up was not released. In the current set-up we can calculate whether or not cerebral accumulated lactate serves as a substrate alternative to glucose. There was no relation between accumulated lactate and VA differences in glucose. Thus, net cerebral blood lactate use is minimal and blood lactate is not a replacement energy substrate for glucose. The 11% lactate taken up by the brain is either not metabolized, or if metabolized, not used for energy consumption (e.g. converted to alanine). These results suggest that there is a lactate pool in the brain that can be filled and emptied depending on the blood lactate concentration, and which is not used as an energy supply. Although we cannot exclude the possibility that high lactate levels may disrupt normal metabolic relationships, our study emphasizes that high lactate does not necessarily lead to altered glucose consumption.

As the venous-arterial method measures net metabolism instead of total metabolism, this conclusion does not necessarily imply that lactate is not metabolized at all. For example, following the injection of  $[3-^{13}\text{C}]\text{lactate}$ , which increased blood lactate concentrations to exercise levels (8-11 mM (Hassel and Brathe, 2000; Bouzier et al., 2000)), rapid incorporation of the label is seen in glutamate, glutamine (Hassel and Brathe, 2000) and alanine (Bouzier et al., 2000), with a cerebral uptake and metabolism of lactate up to 50% that of  $[1-^{13}\text{C}]\text{glucose}$  in awake mice (Hassel and Brathe, 2000). And, although brain energy use is lower under anesthesia (Sibson et al., 1998; Attwell and Laughlin, 2001), lactate is metabolized in the anesthetized rat as well (Bouzier et al., 2000). In addition, lactate metabolism under both pathological and physiological conditions is now well established *in vitro* (Schurr et al., 1988; Schurr et al., 1997; Schurr et al., 1999; Cater et al., 2001). However, incorporation of  $[3-^{13}\text{C}]\text{lactate}$  may overestimate lactate consumption, as the conversion of lactate to e.g. alanine does not necessarily imply lactate metabolism (through the citric acid cycle to  $\text{CO}_2$  and  $\text{H}_2\text{O}$ ). Also, the efflux of unlabeled lactate, which possibly occurs in larger quantities than that of  $[3-^{13}\text{C}]\text{lactate}$ , has not been taken into account in the  $[3-^{13}\text{C}]\text{lactate}$  studies. We believe that  $[3-^{13}\text{C}]\text{lactate}$  experiments are very well suited to study the rapid flux of lactate in the brain, but it does not necessarily imply that lactate metabolism contributes significantly to the net brain energy use. On the other hand, our study could underestimate cerebral lactate consumption, as experiments were performed under halothane anesthesia. Lactate consumption could be higher in conscious animals. Unfortunately, the necessity of anesthesia is a limitation of the present technique.

In conclusion, in this study we have found that lactate does not replace glucose as a cerebral metabolic substrate, and that there is hardly any net lactate use by the brain as 89% of the lactate moving into the brain during infusion is released afterwards and no reduction of glucose consumption is seen at lactate influx. The possible lactate metabolism is of minor importance to total net brain energy consumption. There seems to be a lactate pool in the brain that can be filled and emptied, allowing exchange of lactate even at equimolar concentrations between brain and blood, but which is not used as a supply for cerebral metabolism under normoglycemic circumstances.

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## CHAPTER 8

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